REMARKS

A section titled "Brief Description of the Drawings" has been added. Support can be found throughout the specification, including the drawings, themselves. The claims have been amended in accordance with the examiner's suggestions on Pages 4-5 of the Office action.

Rejection under §103

The activation of the Ras signaling pathway involves a number of interaction steps between the proteins of the EGF receptor/Grb2-Ras fusion protein system.

First, the binding of a ligand to the extracellular domain of the transmembrane receptor EGFR can induce a structural change in the intracellular domain of the receptor.

Second, as a consequence of this structural change, a fusion protein of the claimed invention (which comprises an adaptor polypeptide Grb2 and a Ras polypeptide) can bind to the intracellular domain of the EGF receptor.

Third, as a consequence of this binding, the Ras domain of the fusion protein can be guided on to the cell membrane. The membrane localization of the Ras protein achieved in this manner is a prerequisite for the further activation of the Ras signal transduction pathway in the cell (page 21, lines 23 to 33; Figure 3).

Since the EGF receptor and the Ras polypeptide are human, but the cells used are yeast cells, the interaction which takes place is heterologous. On the basis of the cited prior art, the skilled person could not expect that the heterologous EGF receptor/Grb2-Ras fusion protein pathway would be functionally active in a yeast cell.

The documents Trueheart et al. and Ostanin et al. disclose the introduction of growth factor receptors into cells that are capable of interacting with the cell's endogenous signaling pathway. The two documents do not teach the use of a fusion protein and, in particular, do not

teach the use of a fusion protein which mediates an interaction between the receptor and the signaling pathway.

The Isakoff et al. document appears to disclose a fusion protein comprising a Ras and a PH domain. The fusion protein interacts exclusively with second messenger molecules from P13K which are generated in the cytosol (See, e.g., Abstract). This document also contains data showing that the skilled person cannot predict the interaction of protein domains with certainty. Isakoff et al. are unable to show any *in viro* binding of the PH domain of Sos to second messenger molecules from P13K, even though corresponding *in viro* data are available (See, e.g., paragraph spanning columns 1-2 of page 5380; page 5382, 2nd paragraph). Also, it can only be speculated as to why, surprisingly and in contrast to the PH domains of other proteins (e.g., AKT and BTK), the PH domain of Sos is not capable of such a binding (page 5382, left-hand column).

Aronheim et al. document disclose a fusion protein from a hSos domain and a protein of interest (bait protein, in particular c-Jun). This system is described as being suitable for screening a cDNA library fused to a membrane localization signal.

The Aronheim and Isakoff documents therefore do not disclose a fusion protein which is able to interact with a receptor or in particular the intracellular domain of a transmembrane receptor. Therefore, neither of the two documents contains any indication of an extracellular ligand mediated interaction between a fusion protein comprising a Ras domain and a receptor, in particular an EGF receptor. Thus, none of these documents suggest a cell system which can be used for extracellular ligand screening with a reasonable expectation of success.

An interaction between murine Grb2 as adaptor protein on EGFR expressed by PC12 cells from rat is described in Suen et al. Similarly, Rozakis-Adcock et al. disclose an interaction between human EGFR and endogenous Grb2 in mouse fibroblasts or rat derived cells. Both documents show immunoblotts indicating the respective interactions, but do not present *in vivo* data, which could give a hint that there is an active signal transfer from the EGF receptor to the Grb2 protein. Moreover, an interaction of human EGFR with murine Grb2 in yeast cells is not

disclosed in these documents. Further, the documents contain no indication of a Grb2-Ras fusion protein. Thus, these references provide no expectation that Grb2 fusion proteins would activate a signaling pathway in yeast cells.

In conclusion, none of the cited documents contains an indication that the inventive, non-anticipated, Grb2-ras fusion protein would be suitable for mediating the effect of a binding of an extracellular ligand to the EGF receptor to an endogenous yeast signaling pathway. At the priority date of the present application, therefore, the skilled person could have had no reasonable expectation of success that a heterologous EGF receptor/Grb2-Ras fusion protein system could be functionally active in initiating the Ras pathway in a yeast cell.

The skilled person could also not reasonably expect that a human EGF receptor can exist in a biologically active form in a yeast cell membrane: the receptor must exhibit an active binding site on the exterior of the cell membrane, but in the binding of a ligand, also be able to mediate a structural change in the intracellular domain. The attached document by Lagane et al. contains, for example, the indication that differences in membrane composition found from one cell type to another can represent a limiting factor to recovering the functionality of transmembrane proteins when expressed in heterologous systems.

Since the fusion protein Grb2-Ras is not anticipated by the prior art, the skilled person could also not assume in advance that this artificial construct is biologically active. In contrast, the person skilled in the art of protein chemistry would have been aware of the fact that even small changes concerning the amino acid sequence of a protein can influence the secondary and tertiary structure of a protein respectively. But, the proper tertiary structure is not a prerequisite for the biological activity of a protein. For example, it was not possible in advance to exclude an interference of the two fusion protein domains inhibiting proper folding. Thus, it was not possible to predict that both fusion protein domains according to the invention would fold into a biologically active form.

Furthermore, due to the discussed structural reasons, the skilled person would not assume that, after binding of the fusion protein via Grb2 to the EGF receptor, the Ras domain of the fusion protein would be in a proper spatial arrangement necessary to initiate the further Ras signal pathway of the cell.

Due to the plurality of these unknowns, the skilled person would not expect that the inventive heterologous EGFR/Grb2-Ras fusion protein system in yeast cells is functionally active and, as a consequence of the binding of an extracellular ligand, maintains the Ras activity in the yeast cell according to the invention.

In conclusion, therefore, it is only with inadmissible hindsight that the present invention can be viewed as rendered obvious by the cited prior art documents.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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